THE ACUTE LETHALITY TO RAINBOW

TROUT OF WATER CONTAMINATED

BY AN AUTOMOBILE TIRE

APRIL 1994

⑦ **Ontario**

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ABSTRACT

Rainbow trout fry were exposed to samples of water that had been contaminated by submersing an automobile tire in 300 litres of water. The "tire water" caused 100 % mortality in static acute lethality tests, usually within 48 hours. Three other test species (*Daphnia magna*, *Ceriodaphnia dubia* and fathead minnows (*Pimephales promelas*) were tested but no lethality was observed. Samples of tire water were subjected to benchtop treatments designed to reduce the toxicity and provide some information about the properties of the toxicant. Aeration and the additions of acid, base, an anti-oxidant and a metal chelating agent did not reduce the lethal effects of tire water but activated carbon completely removed the toxicity. Storing samples for seven days under light reduced the toxicity only slightly. Samples of the tire water were analyzed for 143 target compounds. Zinc was the only one found, but the water concentrations were well below lethal levels. To identify nontarget chemicals, gas chromatography-mass spectrometry (GC-MS) scans were performed. Up to 62 organic contaminants were detected in the individual tire water samples, but less than half were identifiable as known compounds. Alkylphenols and aromatic nitrogen compounds were present at low concentrations, but they cannot be eliminated yet as suspected toxicants. The chemical cause(s) of the toxicity to trout remains unconfirmed.

ACKNOWLEDGEMENTS

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1. INTRODUCTION

Every year millions of scrap rubber tires are generated so there is a strong incentive to find new uses for the old tires. Many have proposed the use of tires to build aquatic structures (artificial reefs and floating breakwalls). Laboratory studies (Kellough, 1991; Goudey and Barton, 1992 and Day et al., 1993) report that waters with submersed tires can cause acute lethality to several species of aquatic animals, especially rainbow trout (*Oncorhynchus mykiss*). No chemical cause has yet been identified in water or in animal tissues. The toxicant has been characterized as water soluble, persistent and nonvolatile, hence there is a significant potential for aquatic contamination from tire structures.

A laboratory study was conducted to measure the acute lethality to trout of water that had been contaminated by a submersed tire. The trout tests were conducted on "tire water" before and after it had been subjected to treatments that might reduce or alter the toxicity - a toxicity identification evaluation (Mount and Anderson-Carnahan, 1988). The purpose was to characterize the physical-chemical properties of the toxicant to help narrow down the search for its identity. Three other species (*Daphnia magna*, *Ceriodaphnia dubia* and fathead minnows (*Pimephales promelas*) were tested but no lethality was observed.

2. METHODS

2.1 Toxicity test procedures

The Canadian national procedures were followed for acute lethality testing of rainbow trout and *D. magna* (Environment Canada, 1990a; 1990b). The procedures recommend specifications for animal cultures, dilution water quality, facilities and apparatus, test conditions, observations, measurements and statistical calculations. Acute lethality tests of fathead minnows and *C. dubia* also were conducted. All the tests were conducted from April to July, 1993 under natural indoor light and 16 hours of fluorescent light per day. The temperature, pH, dissolved oxygen concentration and conductivity of test solutions were measured before and after the tests, and mortality checks were made three or four times daily during the tests. Dead animals were removed from the test chambers when first observed. Table 1 summarizes the toxicity testing conditions.

2.2 Culture and Dilution Water

Dechlorinated municipal tap water was used to culture test animals and to prepare and dilute toxicity test solutions. It was an alkaline "hard" water that was temperature-adjusted and aerated to 100 % dissolved oxygen before use. The water quality was chemically characterized as described in Section 2.7.

Table 1. *Summary of the aquatic toxicity tests conditions.*

Test species	trout	*D. magna*	minnow	*C. dubia*
Exposure, hrs.	96	48	96	48
Life stage	fry	neonates	fry	neonates
Wet weight/age	0.7-4.3 g	< 24 hours	0.2-0.4 g	< 24 hours
Test vessel	20 L plastic bucket	50 mL glass vial	250 mL beaker	30 mL plastic cup
Solution volume	20 litres	50 mL	200 mL	15 mL
Loading	10 fish	3 neonates	3 fish	5 neonates
Replicates	none	4	2	2
Temperature, °C	13 to 17	18 to 22	21 to 23	18 to 22
Aeration	yes	no	no	no
Food	none	none	brine shrimp once daily	YCT once at start of the test

2.3 Tire Water

A 6.9 kilogram scrap radial automobile tire was used to contaminate water. Before preparing each water batch, the tire was soaked in water, scrubbed and rinsed with water several times. The tire water was made in a polycarbonate water bath (177 cm long by 66 cm wide). The water bath was filled to a depth of 26 cm with 300 litres of water. The tire was submersed, and the water was continuously and vigorously aerated using a glass air stone and an aquarium air pump. A second water bath without a tire provided the control water.

Three batches of tire water were prepared by submersing the tire for periods of 10 to 14 days. After the submersion periods, samples of the tire waters were collected for toxicity tests, chemical analyses and further physical-chemical treatments. When samples were stored for later testing, they were held as 20-litre aliquots in filled, sealed plastic-lined buckets at 15 °C in the dark. The control water was sampled, treated, stored and tested concurrently with the tire water.

2.4 Animal Cultures

Rainbow trout were from a disease-free hatchery. The trout were acclimated for at least two weeks at 13 to 17 °C before testing. The other species were from reproducing laboratory cultures held at 18 to 22 °C. The two fish species were raised in tanks with aerating flow-through water and the two daphnid species were raised in unaerated static cultures with water replacement two or three times per week. The cultures were fed daily. Rainbow trout were fed commercial trout chow. Fathead minnows were fed live (first and second instar) brine shrimp. *D. magna* were fed a two-algae (*Selenastrum capricornutum* and *Chlorella fusca*) diet. *C. dubia* were fed an aqueous mixture of yeast, cereal grass and trout chow (YCT) supplemented twice weekly with the two-algae diet.

2.5 Toxicity Identification Evaluation

Table 2 is a summary of the following experimental design. Toxicity tests 1a, 1b, 2a, 2b and 2c were conducted on tire water batches 1 and 2 to investigate the sensitivities of the four species. The tests were static except for the fathead minnow test (2c) which had 80 % of the solution volume replaced daily. The animals were exposed to unaltered samples in full dilution series LC50 tests.

Thereafter, single-concentration trout tests using full strength tire water were conducted, and median time to mortality (LT50) was the measure of toxicity. An LT50 also was estimated from the results of test 2a. "Initial" tests (2a and 5) were conducted to ensure the toxicity of the freshly-prepared, unaltered tire water. Chemical additives were mixed into other samples of the same tire water. The chemically-altered samples were held for 24 hours before testing. Each sample was held, altered and tested in the same container. Trout also were exposed to an unaltered sample held for 24 hours in "baseline" tests 3a and 6a to compare to the toxicity of the altered samples.

To investigate the pH-sensitivity of the toxicant, hydrochloric acid and sodium hydroxide solutions (1.0N) were used to make pH 3 and pH 11 tire waters that were readjusted to the initial pH just before toxicity test 3b and 3c. 0.16 g/L ethylenediaminetetraacetate (EDTA) and 0.5 g/L sodium thiosulphate ($Na_2S_2O_3$) tire water solutions were prepared to investigate potential toxicity due to metals in test 3d and oxidants in test 3e. A sample of unaltered tire water was stored for seven days in the dark at 15 °C to evaluate the persistence of the causative agent in test 4.

For toxicity tests 6b, 6c and 6d, acid and base solutions were used to make tire water at pH values of 6, 7 and 8. Activated carbon (10 g/L) was added to an aliquot of tire water for test 6e. Toxicity tests 7a, 7b and 7c were designed to provide a measure of toxicant persistence and sensitivity to light (photolysis). Samples of tire water batch 3 were stored for seven days in the dark at 15 and 20 °C, and under a combination of natural indoor and fluorescent light.

Table 2. *The experimental design.*

Tire water batch #	Toxicity test #	Test species or sample treatment	Sample age days
	LC50 tests of four species		
1	1a	rainbow trout	< 5 hours old
	1b	*D. magna*	< 5 hours old
2	2a	rainbow trout	< 5 hours old
	2b	*C. dubia*	< 5 hours old
	2c	fathead minnow	< 5 hours old
	LT50 tests of rainbow trout		
2	2a	none, initial test	< 5 hours old
	3a	none, baseline test	2
	3b	pH 3	2
	3c	pH 11	2
	3d	EDTA	2
	3e	$Na_2S_2O_3$	2
	4	stored, 15°C, dark	7
3	5	none, initial test	< 5 hours old
	6a	none, baseline test	1
	6b	pH 6	1
	6c	pH 7	1
	6d	pH 8	1
	6e	activated carbon	1
3	7a	stored, 15°C, dark	7
	7b	stored, 20°C, dark	7
	7c	stored, 20°C, light	7

2.6 Statistical Estimates of Toxicity

The concentration-mortality data were used to estimate median lethal concentrations (LC50s) and 95 % confidence limits by the probit method when partial mortalities (16 to 84 %) occurred, or by the Spearman-Karber method when partial mortalities did not occur. The concentration of tire water is given as a percentage by volume. The full strength tire water, described in section 2.3, is the 100 % solution. A 10 % solution would be one part tire water combined with nine parts dilution water. Median lethal times (LT50s) and 95 % confidence limits were estimated similarly using the time-mortality data from the single concentration tests of the full strength tire water. The toxicity estimates for separate samples of tire water were considered different if the confidence limits did not overlap.

2.7 Chemical Analyses

At the same time as tire and control water samples were collected for toxicity testing, water samples also were collected for chemical analyses. The waters were analyzed for copper, nickel, lead, zinc, iron, cadmium, chromium, ammonia, nitrates, nitrite, pH, specific conductivity, dissolved inorganic carbon, dissolved organic carbon, chloride, sulphate, hardness, alkalinity, calcium, magnesium, sodium, potassium and fluoride.

Water samples were analyzed for the concentrations of 143 selected organic compounds. The target compounds include pesticides, hydrocarbons, chlorinated hydrocarbons, polynuclear aromatic hydrocarbons (PAHs), phthalates, ethers, amines and phenols. Most would not be expected in tire water since they are not used in rubber processing and tire manufacture. However, the scrap tire might have sorbed contaminants from roadways, so the possibility of tire surface contamination was investigated, as suggested in Kellough (1991). Samples also were analyzed for "total unfiltered reactive" phenols, a sum parameter measured by the 4-anti-aminopyrine (4-AAP) test.

To identify nontarget organic chemicals in the tire waters, gas chromatography-mass spectrometry (GC-MS) scans were done as "characterization" analyses. The samples were partitioned into base-neutral and acid fractions and extracted with dichloromethane. The reported concentrations are approximate, and were calculated relative to the internal standard, d_{10}-phenanthrene. Clement and Taguchi (1988) discuss the technical limitations of GC-MS and the criteria for the identification of organic compounds.

3. RESULTS

3.1 Tire Water

Water temperatures varied from 14 to 18 °C during the tire submersion periods. The tire and control waters were similar with respect to pH, dissolved oxygen and conductivity. The measured values for all solutions were: 8 to 10 mg/L dissolved oxygen; pH 7.5 to 8.2; 290 to 342 umhos/cm conductivity. The tire water became a pale green colour during the tire submersion periods, while the control water remained clear. The tire surface was coated with a clear "slimey" material after each submersion period, probably due to bacterial growth.

3.2 Toxicity Identification Evaluation

All the tire water batches were acutely lethal to trout. Fish death was preceded by many of the clinical signs listed in McKim et al. (1987) as indicators of a narcotic mechanism of toxic action: Some fish lay on their sides, others were suspended vertically. The fish were

hypoactive and showed very little startle response. Occasional corkscrew spiral swimming was observed and the fish skin colour turned black.

Table 3 gives the concentration-mortality data and the LC50s for the two full dilution series tests. In both tests, the 24-hour LC50 was greater than the 96-hour LC50. Also tire water batch #1 was more toxic than tire water batch #2. Full strength tire water was nonlethal to *D. magna*, fathead minnows and *C. dubia*. No further tests were conducted with these species.

Table 3. *Cumulative percent mortality in the dilution series tests.*

Concentration	Tire water batch #1		Tire water batch #2	
% vol/vol	24 hours	96 hours	24 hours	96 hours
100	100	100	90	100
65	70	100	0	90
40	0	70	0	0
30	0	40	0	0
20	0	0	0	0
10	0	0	0	0
0 (control)	0	0	0	0
LC50	58	34	83	52
Confidence limits	49 to 68	29 to 40	73 to 93	49 to 56

Table 4 gives the trout LT50 estimates for the toxicity identification evaluation. Activated carbon added directly to the tire water test container (test 6e) completely removed the acute lethality to the trout that were added 24 hours later. The same amount of carbon (10 g/L) added to dilution water had no observed effect on the control fish. The EDTA, sodium thiosulphate, acid and base additives did not affect the tire water toxicity, but a slight toxicity reduction did occur with sample storage under light; the fresh samples had the lowest LT50s (tests 2a and 5) while the stored and light-exposed samples had the highest LT50s (tests 4 and 7c). The differences between the LT50s were not large enough to be considered evidence of toxicity reduction.

When the tire water was adjusted to pH 3, the green colour was removed, and it did not reappear when the pH was readjusted back to pH 8. The additions of acid, base and sodium thiosulphate caused increases in tire water conductivity (600 to 770 umhos/cm), due to higher levels of electrolytes. This was not considered to be a stress for the trout because they are euryhaline, and the altered control waters were nonlethal. In the pH 6, 7 and 8 treatments, the pH did not stay at the desired value during the aerating trout exposures, but shifted 0.7, 0.3 and 0.3 units respectively back towards the initial pH (7.5).

Table 4. *LT50s of the single concentration trout tests.*

Sample treatment (toxicity test#)	LT50 (hours)	95 % confidence limits
initial (2a)	17	not calculated
baseline (3a)	23	17 to 28
pH 3 (3b)	25	20 to 29
pH 11 (3c)	25	22 to 30
EDTA (3d)	23	17 to 28
$Na_2S_2O_3$ (3e)	21	15 to 25
stored,15°C,dark (4)	35	27 to 43
initial (5)	18	11 to 22
baseline (6a)	20	14 to 24
pH 6 (6b)	16	not calclated
pH 7 (6c)	18	2 to 22 ·
pH 8 (6d)	18	3 to 22
activated carbon (6e)	nonlethal	not calclated
stored,15°C,dark (7a)	21	18 to 25
stored,20°C, dark (7b)	20	18 to 22
stored,20°C,light (7c)	26	23 to 30

3.3 Chemical Analyses

The tire and control waters were similar with respect to the chemical concentrations of inorganic metals, nitrogen species and general water quality parameters (Appendix 1). The slightly elevated zinc levels in the tire waters were expected because zinc oxides are used in tire manufacture (Fishbein, 1991). None of the other target analytes were found. The detection limits ranged from 1 ng/L to 2 ug/L. The detection limits were well below the known lethal levels of the target compounds, so none were suspected as acute toxicants. Appendix 2 lists the target analytes and their detection limits. The solvent extracts for the target compounds were a bright yellow colour and contained analytical "peaks" that suggested the presence of aromatic nitrogen (arylamine) compounds.

The numbers of compounds detected by the GC-MS scans are given in Table 5. 28 to 62 compounds were detected in the samples, but less than 50 % were identified as specific substances. 2 to 16 compounds were detected in the control water samples. Some tire water compounds were identified as a member of a certain chemical class (Appendix 3). Most of these compounds can be classified as arylamines or phenols, and alkylated forms were the most common structures. This is consistent with the large-volume usage of these chemical classes in rubber processing (Fishbein, 1991).

Table 5. *GC-MS results: the numbers of compounds identified, classified or unknown. The numbers in parentheses refer to the numbers of compounds in the control water samples. 2a and 2b are separate samples collected four days apart.*

Compound category	Tire water batch #			
	1	2a	2b	3
Identified	9 (2)	13 (4)	16 (1)	11 (5)
Classified	10 (4)	4 (0)	9 (0)	10 (3)
Unknown	15 (0)	11 (1)	24 (1)	41 (8)
Total	34 (6)	28 (5)	49 (2)	62 (16)

37 specific organic compounds were identified by the GC-MS analyses of the three tire water batches. 10 compounds occurred only in the control samples, or at approximately equal low levels in both the tire and control samples. They were discounted as laboratory artifacts unrelated to tire rubber contaminants, and they are not shown. The 27 remaining compounds and their approximate concentrations are listed in Appendix 4. The water concentrations were less than 1 ug/L for 18 compounds, and less than 10 ug/L for nine compounds. 4 compounds occurred in all the tire water samples:

aniline
4-(1-methyl-1-phenylethyl)-phenol
benzothiazole
4-(2-benzothiazolythio)-morpholine

0.1 to 0.5 ug/L benzothiazole occurred in three of the four control samples, but these levels were an order of magnitude lower than the concentrations in the tire water samples. Generally, most of the compounds identified by the GC-MS scans have one or more ring structures with nitrogen, sulphur and oxygen groups attached as aromatic substituents, or included in the ring structures as heterocyclic compounds. Again, the prevalent ones belong mostly to the chemical classes (arylamines and alkylphenols) used in large amounts for rubber processing.

4. DISCUSSION

The sensitivity of rainbow trout to the acute lethal effects of tire waters was conclusively demonstrated. Other laboratory studies have reported similar results using different makes and models of tires from several manufacturers. These studies are reviewed:

In Kellough (1991) scrap tires (whole and cut-up into pieces) were submersed in 400 litres of aerating water for 60 days. Samples of the waters were collected after 30 and 60 days for acute lethality tests of rainbow trout and *D. magna*. Other tanks were set-up the same way, and goldfish (*Carassius auratus*) were added for bioaccumulation studies. The 30-day and 60-day tire water caused 100 % mortality to rainbow trout within 24 hours. No cause was identified. The same waters were nonlethal to *D. magna*, and none of the goldfish were killed during the bioaccumulation tests. No chemical contaminant was found in the water or in the goldfish tissues for selected inorganic metals, PAHs and chlorinated aromatic compounds. A GC-MS scan identified 53 organic compounds, mostly nitrogen- and sulphur-containing compounds used in rubber processing and tire manufacture.

In Goudey and Barton (1992), tire water was prepared with 200 g of scrap tire pieces (six makes and models) per litre water. The tire pieces were transferred daily for 65 days to new containers of water and 48-hour single concentration tests of trout, *D. magna* and *C. dubia* were conducted in the new containers with the tire present and in the old containers from which the tire had been removed after a 24-hour submersion period. 90 to 100 % mortality of trout and *C. dubia* was observed in all the tests. *D. magna* were less sensitive and the tire waters varied in toxicity: two types of tires were nonlethal, two caused 60-70 % mortality and two caused 100 % mortality. After 65 days of water changes, the tire waters were still lethal. LC50 tests were conducted using tire water made from pieces of freshly-cut scrap tires and from the tire pieces that had been soaked previously. The trout 96h-LC50s were 10 % and 33 % and the *D. magna* 72h-LC50s were 12 % and nonlethal, respectively. *D. magna* mortality was preceded by immobilization that was not observed until 48 hours of exposure. The authors could not identify the cause, and they concluded, "... the substances released from the tires are water soluble, persistent and have a low volatility."

In Day et al. (1993) tires (new, scrap and from a 10 year-old floating breakwall) were submersed in 300 litres of water for 5, 10, 20 and 40 days, and then acute lethality tests of rainbow trout, *D. magna* and fathead minnows were conducted. The tires from the floating breakwall were nonlethal to trout. All the other tire waters were lethal to trout and the 96h-LC50s were 12 % for scrap tires to 80 % for new tires. No toxicity to the other test species was found. Trout tests were conducted on tire water that had had the tire removed for 1, 2, 4, 8, 16 and 32 days. The tire water remained toxic for up to 8 days for the new tires and 32 days for the scrap tires. The authors concluded, "... tires can continue to release toxic compounds on contact with water for extended periods of time."

In Peterson et al. (1986) chemical analyses were conducted on "tire oil" and downstream water contaminated during an 8-month fire at a tire stockpile. The contaminated water contained benzothiazole derivatives. The oil contained petroleum hydrocarbons as the main components, and the oil was, "highly toxic to all life in the stream...but it is not known which compound or group of compounds caused the toxicity".

In this trout study, the inorganic metals were discounted as toxicants because the chemical analyses found only low, nonlethal levels of zinc, and the toxicity of the tire water was not altered by pH adjustment or by EDTA (a metal chelating agent). The target organic compounds were not found at detection limits well below lethal concentrations. In other laboratory chemical tests, metals were identified in acidic leachate and PAHs in alkaline leachate prepared from tire pieces (Twin City Testing Corporation, 1990).

The toxicity of the tire water was completely removed by the addition of activated carbon, but this treatment removes many contaminants from water so no specific chemical cause is indicated. The toxicant is believed to be an organic compound. Since the tire waters and the trout test solutions were aerated continuously, the toxicant must be nonvolatile. The toxicant was stable in water and is probably a polar (high water solubility) compound, since aging the tire water under light (photolysis) and in plastic containers (a sorbent of nonpolar compounds) did not significantly reduce the toxicity. The increase in percentage mortality over time (96h-LC50 < 24h-LC50) shows that an extended period of time was necessary for the toxicant to partition into the organism and build up to the lethal body burden. This suggests that the toxicant has a relatively high molecular weight (MW) compared to, for example, liquid organic solvents (MW less than about 120 g/mole) that partition rapidly, and thus have constant LC50 values between 24 and 96 hours of exposure. Alternately the delay in toxicity may be due to the time required for trout to metabolically "activate" some precursor chemical to the toxic form.

The organic compounds identified by the GC-MS analyses of the tire waters are known rubber-processing chemicals, impurities and breakdown products. Shaver (1968), Taylor and Son (1982) and Fishbein (1991) discuss the uses of rubber-processing chemicals. Hundreds of compounds may be used in different blends to make synthetic (mostly styrene-butadiene) rubber. The aniline and thiazole derivatives accelerate the rate of vulcanization (cross-linking of rubber polymer chains with sulphur). The accelerator 2-mercaptobenzothiazole (MBT) was not found in the tire waters but 2(3H)-benzothiazolone, 2-(methylthio)-benzothiazole and benzothiazole were prevalent. These compounds are the probable stable end products of MBT formed by methylation, photolysis and oxidation processes (Brownlee et al., 1992). 4-(2-benzothiazolythio)-morpholine is the main ingredient in a commercial blend used as a delayed-action accelerator in rubber processing (Taylor and Son, 1982). The blend contains 2-(methylthio)-benzothiazole as an impurity, and it forms benzothiazole when aqueous solutions are exposed to sunlight (Spies et al., 1987).

Since rubber deteriorates with age because of factors such as oxygen, ozone, oxidation, heat, light and mechanical stress, large amounts of additives are used to protect finished products. The arylamines and the alkylphenols in the tire waters were probably antioxidant and antiozonant additives. The ketones could have originated from the additives or from the oxidation of the rubber, known to form compounds with oxygen-containing groups such as keto, alcohol and carboxyl compounds that are found in all aged rubbers. The formamide compounds were probably from solvents used in rubber processing. 2,5-cyclohaxadiene-1,4-dione (p-benzoquinone) was likely an oxidation product of aniline or hydroquinone.

Little is published in the literature about the aquatic toxicity of the identified compounds or their derivatives. 23 mg/L [1,1-biphenyl]-2-amine caused 50% population growth inhibition of the ciliate protozoan *Tetrahymena pyriformis* (Schultz et al., 1989). The acute LC50 values for aniline toxicity to rainbow trout range from 10 to 40 mg/L (Bazinet, 1990). Verschueren (1983) lists LC50s of 5 to 10 mg/L p-benzoquinone for an unspecified exposure of perch, and "approximate fatal concentrations" of 0.28 mg/L hydroquinone and 6 mg/L p-phenylenediamine for 48-hour exposures of goldfish.

The acute lethal levels of most water soluble organic chemicals (typically 100 ug/L to 100 mg/L) are much higher than the approximate concentrations of the compounds identified here (less than 10 ug/L). Even though the reported concentrations were low, the GC-MS-identified compounds cannot be eliminated as suspected toxicants for several reasons. If the toxicant were not extracted efficiently by the dichloromethane solvent, only a fraction of the original concentration might be found. Since d_{10}-phenanthrene (a three-ring PAH) was the analytical standard for the GC-MS scans, the technique cannot be expected to provide a quantitative measure of all the diverse chemical structures that were detected. Some compounds might have been destroyed in the analyses. For example, one would expect to find n-nitrosodiphenylamine in tire water because large amounts are used to control vulcanization during rubber processing. But the nitrogen group is known to be cleaved off by the high temperatures used in gas chromatography, leaving diphenylamine (N-phenyl-aniline) that was found by the GC-MS scans.

Brownlee et al. (1981) discusses the properties of benzothiazoles in relation to their aquatic environmental fate. The benzothiazoles might be suspected as the toxicants because they were prevalent, but they are too volatile compared to the toxicant. Their physical-chemical properties suggest that their toxic effect would occur more rapidly than was observed for the tire waters. The water solubility (C_s) and octanol-water partition coefficients (Kow), reported by Brownlee et al. (1992), are: benzothiazole C_s = 3000 mg/L, log Kow = 2.0; 2-(methylthio)-benzothiazole C_s = 125 mg/L, log Kow = 3.1. The contribution of benzothiazoles and other organic sulphur compounds to the tire water toxicity could be investigated by adding nonlethal amounts of a mercury salt to the tire water to bind the sulphur compounds and thus reduce their bioavailability and toxicity.

The only chemical measurement associated with the toxicity was "total phenolics": 9 ug/L in tire water batch #2 that was lethal to trout, and 4 ug/L in a nonlethal sample of tire water. The nonlethal tire water was made using the same conditions described above except that the tire had been used previously for a separate unreported sublethal test of rainbow trout liver enzyme induction (This was a 12-day submersion of the tire in 600 litres of water flowing at 1 litre per minute with five 200-gram rainbow trout inhabiting the tire water). Too few data were available to attempt a concentration-lethality correlation. Even if a strong correlation were found, this would not prove causality without additional experimental evidence (Mount, 1988). Unfortunately this chemical sum parameter does not adequately measure substituted phenols with alkyl, aryl, nitro, benzoyl, nitroso or aldehyde groups in the para position. Substitution generally reduces chemical recovery, and thus the reported values represent the minimum concentrations. Nevertheless, large amounts of substituted phenols are used in rubber processing, and they are water soluble and nonvolatile like the toxicant. Alkylphenols in particular are exceptionally toxic, primarily due to their ability to affect lipid structures such as cell membranes (Curvall et al., 1984).

The ayrlamines also remain as suspected toxicants. As a group they are used in large amounts for rubber processing, and they have been identified as a major cause of human health effects in the rubber industry (Fishbein, 1991). Phenols and arylamines both cause fish toxicity by a polar narcosis mechanism (Schultz et al., 1989), consistent with the narcotic signs observed for the trout exposed to tire water. In water, arylamines are known to be stable, and they are more soluble than their nonsubstituted or sulphur-containing analogs.

Finally, it is possible that a combination of organic contaminants each at a low concentration, could "add up" to cause mixture toxicity. Certainly the literature on narcotic toxicity has demonstrated this for combinations of up to 50 organic chemicals each at levels as low as 0.25 % of the LC50 (Deneer et al., 1988) and for sub-threshold mixtures of compounds from different chemical classes (Broderius and Kahl, 1985).

5. CONCLUSIONS

The observed toxicity to trout is consistent with the results reported in the literature for a variety of different tires but the chemical cause remains unknown. Phenolic compounds and nitrogen aromatic and heterocyclic compounds should be investigated further. Other laboratory studies show that when the amount of tire material in water is five or six times greater than was used here, other aquatic species can be adversely affected.

A separate unreported experiment showed no lethality after the tire had been submersed in a large volume of flowing water containing rainbow trout. Perhaps the tire had a finite amount of leachable toxicant that had been "diluted away" by the 17,280 litres that had

passed over the tire, or the composition of the rubber at the tire surface had changed over time, or the toxicant leaching was blocked by a surface barrier of the bacterial "slime" that coated the tire. In aquatic systems, the tires could become coated with a variety of attached organisms like bacteria, algae and mussels that would block the toxicant from dissolving in ambient surface waters. Also degradation processes like photolysis and microbial activity might be greater in surface waters than in the laboratory and could act as detoxification processes. Field observations, noted in the literature discussed above, indicate that tires are successfully used as habitat by fish and other aquatic life. To follow-up this work, acute lethality trout tests should be conducted on the following types of tire waters:

1.   prepared with tires collected from aquatic structures of various ages to find out how much submersion time in natural waters is required before the tires are rendered nonlethal. *In situ* toxicity tests placing cages of trout beside tire structures of different ages would also be useful.

2.   prepared with scrap tires submersed in metered, flowing water to measure the rate and amount of toxicant released from tires.

3.   prepared with scrap tires to continue the tire water treatments that characterize, identify and confirm the causative agent(s).

The toxicant was relatively persistent and nonvolatile and is thought to be some rubber-processing chemical or mixture of chemicals dissolving from the rubber into water. Surface contamination of the tire was unlikely to be the cause since no "priority pollutants" were detected, and the tire remained toxic after the surface was scrubbed and after a combined total of 40 days of submersion. The total phenolics analysis is recommended as a "marker" of water contamination by tire material because of the large-volume usage of phenolic chemicals in rubber processing, their detectable occurrence at a higher level in toxic tire water than in non-toxic tire water, and their lack of volatility like the toxicant.

Since the toxicant was persistent and nonvolatile, accumulations in sediments are possible, and should be investigated. Other sources of related aquatic contamination that should be considered include: effluents from rubber processing and tire manufacturing plants, leachate from shredded rubber used as roadfill and from tires buried in dump sites, runoff from tire storage/disposal sites and road runoff of contaminants from rubber particles worn off of tire treads.

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APPENDIX 1

THE CHEMICAL ANALYSES OF TIRE WATERS AND CONTROL WATERS

Water concentrations are mg/L. The nitrogen compounds as N. Carbon = dissolved carbon as C. UNF = unfiltered, R = reactive, T = total. ND = not detected. For nondetectable parameters, the detection limits were: ammonium = 0.05; nitrite = 0.015; copper = 0.0019; nickel = 0.004; lead = 0.008; zinc = 0.007; cadmium = 0.0002; chromium = 0.002.

Chemical parameter	Tire water batch #		Control water batch #	
	2	3	2	3
General water quality				
hardness, T, as $CaCO_3$	152	147	151	147
calcium, UNF,R	45	44	45	44
magnesium, UNF,R	10	9	10	9
sodium, UNF,R	14	14	14	14
potassium, UNF,R	1.7	1.7	1.7	1.7
alkalinity, T, as $CaCO_3$	99	98	98	99
fluoride, UNF,R	1.2	1.2	1.2	1.2
chloride, UNF,R	28	28	27	29
sulphate, UNF,R	32	33	31	33
carbon, organic	3	2	2	2
carbon, inorganic	20	22	18	23
Nitrogen compounds				
nitrogen, Kjeld., UNF,R,T	0.25	0.40	ND	0.30
ammonium, fraction R,T	ND	0.40	ND	0.25
nitrates, fraction R,T	0.45	0.45	0.45	0.45
nitrite, fraction R	ND	0.030	ND	0.035
Inorganic metals (UNF,T)				
copper	0.002	ND	0.003	0.0022
nickel	ND	ND	ND	ND
lead	ND	ND	ND	ND
zinc	0.025	0.023	ND	ND
iron	0.06	0.03	0.04	0.07
cadmium	ND	ND	ND	ND
chromium	ND	ND	ND	ND

APPENDIX 2

TARGET ANALYTES AND DETECTION LIMITS
(None were found in the tire water)

Gasoline and solvents, ug/L

1,2-dichloroethylene	2	m-, p-xylenes	2
1,1-dichloroethane	2	bromoform	2
chloroform	2	1,4-dichlorobenzene	2
1,1,1-trichloroethane	2	1,3-dichlorobenzene	2
carbon tetrachloride	2	1,2-dichlorobenzene	2
benzene	2	$BCl_2CH + C_2Cl_3H$	2
1,2-dichloropropane	2	$C_2CL_4 + ClBCH_2$	2
toluene	2	o-xylene	2
1,1,2-trichlorethane	2	1,1,2,2-tetrachloroethane	2
chlorobenzene	2	gasoline	1
ethylbenzene	2	fuel distillate	1

Pesticides, ug/L

ametryne	0.05	difenoxuton	2
atrazine	0.05	diuron	2
prometryne	0.05	fluometuron	2
atrazone	0.05	linuron	2
metolachlor	0.5	metoxuron	2
diethyl simazine	0.5	monolinuron	2
chlorbromuron	2	monuron	2
chlortoluron	2	neburon	2
siduron	2		

APPENDIX 2

TARGET ANALYTES AND DETECTION LIMITS
(None were found in the tire waters)

Organochlorines, ng/L

hexachloroethane	1	alpha-BHC	1
1,3,5-trichlorobenzene	5	beta-BHC	1
1,2,4-trichlorobenzene	5	gamma-BHC	4
hexachlorobutadiene	1	a-chlordane	2
1,2,3-trichlorobenzene	5	g-chlordane	2
2,4,5-trichlorotoluene	5	oxychlordane	2
2,3,6-trichlorotoluene	5	op-DDT	5
1,2,3,5-tetrachlorobenzene	1	pp-DDD	5
1,2,4,5-tetrachlorobenzene	1	pp-DDT	5
2,6,alpha-trichlorotoluene	5	methoxychlor	5
1,2,3,4-tetrachlorobenzene	1	heptachlorepoxide	1
pentachlorobenzene	1	endosulfan I	2
PCB, total	20	dieldrin	2
hexachlorobenzene	1	endrin	5
heptachlor	1	endosulfan II	5
aldrin	1	endosulfan sulphate	5
pp-DDE	1	octachlorostyrene	1
mirex	5	toxaphene	500

Acid extractables, ug/L

phenol	0.2	2,4,5-trichlorophenol	0.2
2,4-dimethylphenol	2	2,3,4-trichlorophenol	0.2
p-chloro-m-cresol	0.2	2,3,5,6-tetrachlorophenol	0.2
2,4-dichlorophenol	0.2	2,3,4,5-tetrachlorophenol	0.2
4-nitrophenol	0.5	pentachlorophenol	0.2
2,3,4,6-tetrachlorophenol	0.2	2,6-dichlorophenol	0.2
2,4-dinitrophenol	20	m-cresol	0.2
4,6-dinitro-o-cresol	10	o-cresol	0.2
2-chlorophenol	0.2	p-cresol	0.2
2,4,6-trichlorophenol	0.2	2,3,5-trichlorophenol	0.2

APPENDIX 2

TARGET ANALYTES AND DETECTION LIMITS
(None were found in the tire water)

Polynuclear aromatic hydrocarbons, ng/L

phenanthrene	10	perylene	10
anthracene	1	benzo(k)fluoranthene	1
fluoranthene	20	benzo(a)pyrene	5
pyrene	20	benzo(g,h,i)perylene	20
benz(a)anthracene	20	dibenz(a,h)anthracene	10
chrysene	50	indeno(1,2,3-c,d)pyrene	20
dimethyl benz(a)anthracene	5	benzo(b)chrysene	2
benzo(e)pyrene	50	coronene	10
benzo(b)fluoranthene	10		

Base-neutral extractables, ug/L

diphenyl ether	0.2	bis(2-chloroethoxy) methane	0.2
bis-2-ethylhexylphthalate	1	naphthalene	0.2
di-n-octylphthalate	0.2	acenaphthylene	0.2
benzo(k)fluoranthene	0.2	2,6-dinitrotoluene	0.5
benzo(a)pyrene	0.2	acenaphthene	0.2
indeno(1,2,3-cd)pyrene	0.5	4-chlorophenyl phenyl ether	0.2
diphenylamine	2	2,4-dinitrotoluene	0.5
3,3-dichlorobenzidine	1	fluorene	0.2
bis(2-chloroethyl)ether	0.5	n-nitrosodiphenylamine	2
indole	0.2	4-bromophenyl phenyl ether	0.2
dibenzo(a,h)anthracene	0.5	phenanthrene	0.2
benzo(g,h,i)perylene	0.2	anthracene	0.2
bis(2-chloroisopropyl)ether	0.2	di-n-butylphthalate	0.2
fluoranthene	0.2	1-methylnaphthalene	0.5
pyrene	0.2	2-chloronaphthalene	0.2
butylbenzylphthalate	0.5	2-methylnaphthalene	0.2
benzo(a)anthracene	0.2	5-nitroacenaphthene	1
chrysene	0.2	benzo(b)fluoranthene	0.2
camphene	0.5	biphenyl	0.2
n-nitrosodi-n-propylamine	0.5	perylene	0.5
1-chloronaphthalene	0.2		

APPENDIX 3

THE COMPOUNDS IDENTIFIED AS MEMBERS OF CERTAIN CHEMICAL CLASSES

The numbers in parentheses refer to the numbers of compounds detected in the same chemical class.

Tire water batch #			
1	**2a**	**2b**	**3**
a methyl-aniline	a methyl-aniline	a methyl-aniline	a methyl-aniline
a nitrogen compound	a nitrogen compound (2)	an amine (2)	a dimethyl-aniline
a C_9 alkylphenol	a phenol	a methyl diphenyl-amine	a methyl diphenyl-amine
a chloro-dimethyl phenol		a C_{10} alkylphenol	a C_4-alkyl pyridine
a phenol (2)		a chloro-dimethyl phenol	a nitrophenol
a chlorine compound		a C_3 alkyl-benzene	a chloro-dimethyl phenol
an alcohol or ether (3)		a carboxylic acid	a methyl phenyl-ethylphenol
		a carboxylic acid ester	a dihydro-indenone + phenol
			a carboxylic acid (2)

APPENDIX 4

THE IDENTIFIED COMPOUNDS AND CONCENTRATIONS (UG/L) IN THE TIRE WATERS

Compound name	CAS #	Tire water batch #			
		1	2a	2b	3
aniline	62-53-3	3	0.3	0.9	0.5
2-methyl-N-phenyl-aniline	1205-39-6	1	1		
N-phenyl-aniline	122-39-4			0.5	0.5
N,N-diphenyl-1,4-benzenediamine	74-31-7			0.4	
[1,1-biphenyl]-2-amine	90-41-5		0.8		
N,N-dibutyl formamide	761-65-9			0.2	
diphenyl formamide	607-0-1			1	
N-phenyl-formamide	103-70-8			0.5	0.3
N,N-diphenyl-hydrazinecarboxamide	603-51-0	0.8			
phenoxazine	135-67-1		5		
benzothiazole	95-16-9	9	1	10	9
2-(methylthio)-benzothiazole	615-22-5		0.4		0.3
2(3H)-benzothiazolone	934-34-9	4	0.1	4	
4-(2-benzothiazolylthio)-morpholine	102-77-2	6	2	5	3
4-acetyl morpholine	1696-20-4		0.7		
2,5-cyclohexadiene-1,4-dione	106-51-4				0.3
1,4-cyclohexanedione	637-88-7	0.3	0.5	0.7	
2,3-dihydro-1H-inden-1-one	83-33-0			0.2	
4-methyl-2-pentanone	108-10-1			1	
1-methyl-2-pyrrolidinone	872-50-4				0.2
4-(1-methyl-1-phenylethyl)-phenol	599-64-4	0.1	0.6	0.8	0.6
4-(phenylamino)-phenol	122-37-2				8
dinitro propyl phenol	1420-07-1			0.6	
2,6-bis(1,1-dimethylethyl)-4-ethyl phenol	4130-42-1		0.7		

Compound name	CAS #	Tire water batch #			
		1	2a	2b	3
2-chloroethanol phosphate	115-96-8			0.2	
9H-fluoren-9-ol	1689-64-1				0.3
α,α-dimethyl benzenemethanol	617-94-7	0.3	0.3	0.4	